Exhibit 1 - Report of First Clinical Trial

Acambis

CLINICAL STUDY REPORT

Phase 1, Dose Escalation, Safety and Immunogenicity of Two New Attenuated Vaccine Strains for Enterotoxigenic *E. coli* in Volunteers

Inpatient Study

(Protocol# VTU983)

BB-IND#: 7922

Sponsor: David A. Sack, M.D.
A. Louis Bourgeois, Ph.D.
The Johns Hopkins University

Financed by:
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Study Start Date: October 27, 1998	Study Completion Date: March 9, 1999	Date of Report: October 22, 2001

FINAL REPORT

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GLOSSARY OF ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
ALS	Antibody from lymphocyte supernatant
ASC	Antibody secreting cells
CTB	Cholera toxin B subunit
CFA	Colonization factor antigen
CFR	Code of Federal Regulations
CMI	Cell Mediated Immunity
CRF	Case Report Form
CT	Cholera Toxin
E.COLI	*Escherichia coli*
ELISA	Enzyme-linked immunosorbent assay
ETEC	Enterotoxigenic *E. coli*
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GCRC	General Clinical Research Center
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IRB	Institutional Review Board
LT	Heat-labile enterotoxin from *E. coli*
ML	Milliliters
PBML	Peripheral blood mononuclear lymphocytes
SAE	Serious Adverse Event
ST	Heat-stable toxin
µg	Microgram
VTU	Vaccine testing unit

1 SYNOPSIS

Name of Sponsor / Company: David Sack, M.D. and transferred to A. Louis Bourgeois, Ph.D. (Johns Hopkins University) / Acambis, Inc.	**Individual Study Table Referring to Part of the Dossier** **Volume:**	**(For National Authority Use only)**
Investigational product: Enterotoxigenic *E. coli* (ETEC) vaccine PTL-ETEC-002 and PTL-ETEC-003		
Active ingredient: Live fresh washed ETEC bacteria	**Page:**	

Title: Phase 1, Dose Escalation, Safety and Immunogenicity of Two New Attenuated Vaccine Strains for Enterotoxigenic *E. coli* in volunteers

Investigators: David Sack, M.D. (PI)

Study centre: General Clinical Research Center at Johns Hopkins Hospital, Baltimore, MD

Analytical site: Clinical Laboratory - Johns Hopkins Hospital and Research Microbiology - Department of International Health in the Johns Hopkins University Bloomberg School of Public Health

Study Period of clinical phase: **Date first volunteer admitted for enrollment (vaccination):** 26 October 1998 **Date first volunteer enrolled (vaccinated):** 27 October 1998 **Date last volunteer completed:** 09 March 1999 (14 days post last vaccination)	**Clinical Phase:** I

Objectives:

- To recruit volunteers according to the protocol's inclusion and exclusion criteria, to admit these volunteers to the General Clinical Research Center (GCRC) and give them a dose of vaccine candidate, to monitor them clinically and manage any symptoms which might occur.
- To monitor the fecal excretion of the vaccine candidate strains.
- To measure the serological response as determined by the antibody titers in the serum and the supernate of lymphocytes cultured *in vitro*.

Methodology: Single-center, open-label, inpatient, safety and immunogenicity study to evaluate two new attenuated strains of enterotoxigenic *E. coli* vaccine (PTL-ETEC-002 and PTL-ETEC-003). Vaccine was administered orally as a single dose on Day 0 to eligible inpatient volunteers. Both vaccine strains were given in a dose-escalation design with escalation to the higher dose level dependent on lack of clinically significant effects at the lower dose level; the planned escalation was from 5×10^7 bacteria to 5×10^9 bacteria to 5×10^{10} bacteria.

Number of subjects (*planned and analys d*): 30 planned (15 for each strain; PTL-ETEC-002 and PTL-ETEC-003), 3 each to receive 5 x 10^7 bacteria, 6 each to receive 5 x 10^9 bacteria, and 6 each to receive 5 x 10^{10} bacteria. A total of 27 volunteers in six groups received vaccine, a total of 6 in the 5 x 10^7 bacteria group, 11 in 5 x 10^9 bacteria group, and 10 in 5 x 10^8 bacteria group.
Diagnosis and main criteria for inclusion: Healthy, non-immunocompromised, male or female inpatient volunteers, >18 or <50 years of age with none of the following: clinically significant medical history, physical examination, or screening laboratory examinations (complete blood count with differential, blood chemistry, urinalysis), negative serologies for HbsAg, HCV, HIV, negative urine HCG within 4 days of immunization (women only), and volunteers over the age of 40 with a normal EKG. Volunteers were required to complete a training session, provide written informed consent, and demonstrate comprehension of the protocol procedures and knowledge of diarrhea and ETEC bacteria, by passing a written examination. Volunteers were excluded from the study if they had a chronic illness, regular use of laxatives or abnormal stool pattern, travelled to a developing country within 5 years, previously participated in an ETEC study, or if antibiotics were used within 7 days of vaccination.
Test product, dose and mode of administration, batch number: Sequential groups of volunteers were to receive oral doses of 5x10^7, 5x10^9 or 5x10^{10} CFU of freshly grown bacteria. Subjects received 120ml of buffer (sodium bicarbonate solution [1.33% w/v in water]) to neutralize stomach acid, followed one minute later with vaccine suspended in 30ml of the same sodium bicarbonate solution.
Reference product: none
Duration of study drug treatment: Single oral administration of one of 3 dose levels of vaccine.
Criteria for Evaluation: **Safety:** Reactogenicity was ascertained by analysing documented signs and symptoms of illness where the hospitalized subject was monitored twice daily on the day of immunization and for the 6 days after immunization. Vital signs including heart rate, blood pressure, respiratory rate and temperature, were performed three times a day while hospitalized. Also documented, was the date, grade, and the weight of all stools passed during this hopitalization period; the first two stools were collected daily and sampled for microbiological examination and assessment of bacterial shedding and occult blood. Fluid intake and output were measured during this hopitalization period. If no symptoms developed following vaccination, on Day 4 subjects were given Ciprofloxacin 500 mg for three days. If subjects developed symptoms prior to Day 4, antibiotic treatment could be initiated at the Investigator's discretion. Subjects were to be discharged on Day 6. After hospital discharge, subjects were asked to contact the Vaccine Testing Unit in the event of late symptoms. The subjects were asked to return to the outpatient clinic on days 10 and 14 for to ascertain intervening medical history.

Immunogenicity:
The immunogenicity of the vaccine was evaluated by antibody response to the vaccine strains and to IgG and IgA antibody to CFA II. Blood samples for serology (serum and lymphocyte specimens) were to be collected prior to vaccination, on Day 9 and Day 14 after vaccination according to schematic and protocol but samples were obtained prior to vaccination, and on Days 7, 10 and 14.

Statistical methods: This sample size did not allow for statistical methodology. Adverse events were summarized by frequency of occurrence, number of subjects experiencing adverse events, severity and relationship to investigational vaccine. Immune response to the vaccine was determined qualitatively without pre-study definitions of positive and negative responders.

Safety results:
No serious vaccine related adverse events were reported. No clinically significant trends in adverse events, vital signs or screening clinical laboratory test were observed in regard to subject safety.
Six (6) volunteers received 5 x 10^7 per dose of strain PTL-ETEC-002 (N=3) and PTL-ETEC-003 (N=3). No significant adverse events were seen and the study proceeded to the next highest dose group 5 x 10^9. Eleven (11) volunteers received 5 x 10^9 (N=5 for strain PTL-ETEC-002 and N=6 for strain PTL-ETEC-003) and adverse events including moderate gas/cramps (N=3), one episode of vomiting, and two cases of grade 3 diarrhea were seen at this dose group. Therefore the next dose group received a reduced dose, 5 x 10^8 per dose strain. Ten (10) volunteers received 5 x 10^8 (N=6 for strain PTL-ETEC-002 and N=4 for strain PTL-ETEC-003) and two cases of moderate gas/cramps and one episode of vomiting were noted. **Table 7** delineates the incidence of symptoms per dose group. None of the volunteers developed an elevated temperature. In neither case of diarrhea, vomiting or gas/cramps did the volunteers require restricting or changing activities.

Efficacy results:
Excretion of vaccine strains: Among those study subjects receiving a dose of 5 x 10^7 CFU, the vaccine was recovered from the stools of all of 6 volunteers at some time. It was recovered the same day as vaccination from two volunteers and continued to be excreted for up to four days in two volunteers. Of those who received the 5 x 10^8-dose level, 9 of 10 volunteers excreted the vaccine strain at some time, but one volunteer never excreted the strain. Again, some volunteers continued to excrete for up to four days. Of those who received a dose of 5 x 10^9, all of 11 volunteers excreted the vaccine strain and all continued to excrete for four days, compared to only 4 of 16 who received lower doses who continued to excrete for four days ($p<0.0001$, Fisher's Exact Test). There was no difference in the frequency or duration of the excretion of the two vaccine strains when given at comparable doses.

Serology results:
Immune response to the vaccine was assessed by determining serum antibody levels at various times (7, 10 and 14 days) following vaccination compared to baseline values. Immune response was also assessed by a modified antibody secreting cell assay (ALS, antibody lymphocyte supernatant assay) in which peripheral blood monocytes sampled 7 and 10 days following immunization were cultured and their supernant fluids assayed by ELISA for antigen specific antibodies. The titers of serum IgG and IgA anti-CFA did not change significantly between the sample collected prior to vaccination and those collected after vaccination. It was noted that there was great variability between the titers from one volunteer to others. Titers of anti-IgG and IgA from the ALS specimens increased significantly between the preimmune specimen collected on Day 0 and the specimen collected on Day 7 after vaccination. By Day 10-post vaccination, the titers decreased.

Conclusion: The vaccine strains were associated with mild and moderate symptoms by protocol and/or case report form definition. Neither IgG nor IgA serum anti-CFA I antibody responses were detected in any of the volunteers. Anti-CFA responses were seen in the specimen from the ALS specimen, which peaked on Day 7-post vaccination and returned to near baseline by Day 10-post vaccination.

These data suggest that both vaccine strains are safe and form a basis for further evaluation of PTL-ETEC-002 and PTL-ETEC-003 in the outpatient study. The outpatient study should include an assessment of duration of excretion and a control group to better assess the relation of symptoms with the vaccines. The lymphocyte antibody response (ALS) should also be continued in the outpatient study, as it appeared to be a more sensitive assay for measuring vaccine induced anti-CFA/II immune responses than serum antibodies. The follow-on outpatient study should also be used as an opportunity to compare the ALS and antibody secreting cell assay (ASC), as measures of vaccine-induced mucosal immune responses.

REPORT SIGNATURES

Our signature(s) below confirm the accuracy and content of the data contained within this report and our respective analyses and summaries thereof:

Investigator:
A. Louis Bourgeois, Ph.D.

Signature _____ Date _____

Medical Monitor:
William B. Greenough, M.D.

Signature _____ Date _____

Authors of Report:
Maria Berkheimer, M.S.,
The Total Approach, Inc.

Signature _____ Date _____

Cynthia K. Lee, Ph. D.,
Acambis Inc.

Signature _____ Date _____

2 ETHICS

2.1 Institutional Review Board (IRB)

Prior to implementation, the study protocol was approved, in writing, by the IRB of the Johns Hopkins University School of Medicine or Johns Hopkins Hospital. All subject-related procedures were carried out at the General Clinical Research Center (GCRC) of the Johns Hopkins Hospital, Baltimore, MD.

IRB membership was maintained according to federal guidelines set forth in CFR Part 56. Details of the constitution of the IRB, including the names of their Chairs, are held on file at the Vaccine Testing Unit (VTU), The Johns Hopkins Bloomberg School of Public Health. For a copy of the IRB approval details refer to **Appendix 12.4**.

This study was conducted under an IND (BB-IND#7922, Serial #001) held by David A. Sack, M.D. and later transferred along with the investigator responsibilities to A. Louis Bourgeois, Ph.D., The Johns Hopkins University. The study was financed by Acambis Research Limited previously known as Peptide Therapeutics Limited.

2.2 Ethical Conduct of the Study

The study was carried out in accordance with the ethical principles outlined in the Declaration of Helsinki, 1964 and subsequent amendments.

2.3 Subject Information and Consent

In response to advertisements published in local papers (see example in **Appendix 12.4**), subjects interested in participation contacted the VTU and were invited to attend a briefing at the VTU at which the study was outlined. Written information/consent forms were given to the subject to study. For participants at VTU, there were separate consent forms for the collection of screening blood samples and for study participation. Examples of all consent forms are given in **Appendix 12.2**. Subjects interested in participation were invited back to VTU for the collection of screening samples. Witnessed written informed consent was obtained by study personnel, prior to any study-related procedure. Enrollment to the trial took place a few days prior to, or on the day of the first vaccination. For eligible subjects, enrollment comprised a final briefing by one of the study personnel, and a written comprehension test (in which subjects were required to score at least 70%).

3 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The following key personnel from the VTU were involved in the management of the inpatient study and the subjects enrolled:

Medical Monitor: William B. Greenough, M.D.

Principal Investigator: David A. Sack, M.D.

Sub-investigator: Janet Shimko, R.N.

Statistician: No formal statistics performed. Data listings and tabulations were compiled.

Study Coordinator: Janet Shimko, R.N.

Study Nurse: Janet Shimko, R.N.

Curricula vitae for key personnel are located in **Appendix 12.5**.

The Study Drug was administered by David A. Sack, M.D.

Johns Hopkins Hospital Laboratory, 600 Wolfe Street, Baltimore, MD 21205 performed clinical chemistry and routine hematology assays.

The following personnel were responsible for the bacteriological and immunological assays: David Sack, M.D. and George Gomes.

Management of the clinical data (i.e. all data with the exception of those derived from the bacteriological and immunological assays) was carried out by Vaccine Testing Unit: David Sack, M.D. and Janet Shimko, R.N.

4 INTRODUCTION

Enterotoxigenic *Escherichia coli* (ETEC) is the major etiological agent associated with traveller's diarrhea in many parts of the developing world and is a major cause of morbidity in both military and civilian travellers to these regions[1]. It also causes up to 380,000 deaths in infants and young children in endemic regions.

There is currently no licensed vaccine available for the prevention of ETEC disease, although there is a candidate vaccine being developed by Powderject, which is currently undergoing phase III evaluation. This consists of an inactivated whole cell preparation of five different ETEC strains, combined with recombinant cholera toxin B subunit (CT-B), which is administered as two oral doses.[2, 3, 4, 5]

The vaccines tested in this study consisted of live attenuated strains of ETEC for oral delivery. Similar live attenuated bacterial vaccines have been developed against *Salmonella typhi* and *Vibrio cholera*. Live attenuated ETEC organisms colonise the intestinal mucosa of vaccinees, providing prolonged exposure to antigen, and will avoid the need for the addition of exogenous adjuvant. It is hoped that a single dose of vaccine will prove to be effective.

ETEC pathogenicity is well understood; fimbrial Colonisation Factors (CFA) mediate adherence to the surface of the intestinal epithelium where the bacteria secrete enterotoxins, which are responsible for the debilitating watery diarrhea. Protective immunity requires both a secretory IgA response against the CFA to block adherence and toxin neutralising antibodies.[6, 7, 8, 9]

A spontaneous toxin deletion mutant of a diarrheagenic ETEC strain (E1392/75/2A) had previously been isolated and tested in phase I studies as a potential vaccine.[8] This is a CS1, CS3 expressing CFA/II strain of the O6:H16 serotype. While providing significant protection against challenge in volunteers, it still caused low-grade diarrhea in 15% of recipients. To further attenuate this strain, two deletion mutations were introduced into the chromosome of E1392/2A. The first strain (PTL-ETEC-002) is deleted in *aroC* and *ompR* genes and the second strain (PTL-ETEC-003) is deleted in *aroC, ompC* and *ompF*. *AroC* is the gene encoding chorismate synthase in the aromatic amino acid biosynthetic pathway. *OmpR* encodes a regulatory protein which controls the inverse regulation of *ompC* and *ompF*, encoding outer membrane porins expressed at high and low osmotic pressure, and certain other genes including those responsible for the expression of Vi antigen in *S. typhi*. Phenotypically both sets of mutations are expected to reduce the ability of the organism to adapt to the conditions in the human digestive tract, attenuating its ability to colonise and cause disease.

5 STUDY OBJECTIVES

To recruit volunteers according to the protocol's inclusion and exclusion criteria, to admit these volunteers to the General Clinical Research Center (GCRC) and give them a dose of either vaccine candidate, to monitor them clinically and manage any symptoms which might occur.

To monitor the fecal excretion of the vaccine candidate strains.

To measure the serological responses to each vaccine candidate as determined by the antibody titers in the serum and the supernate of lymphocytes cultured *in vitro*.

6 INVESTIGATIONAL PLAN

6.1 Overall Study Design and Plan-Description

The trial was designed as a 30 subject (15 for each vaccine), single-center, open-label, inpatient, safety and immunogenicity study to evaluate two new attenuated strains of enterotoxigenic *E. coli* vaccine (PTL-ETEC-002 and PTL-ETEC-003) given in a dose-escalation design with escalation to the higher dose level dependent on lack of clinically significant effects at the lower dose level, the planned escalation was from 5×10^7 bacteria to 5×10^9 bacteria to 5×10^{10} bacteria. The protocol and the case report forms are attached in **Appendices 12.1** and **12.3** respectively.

6.2 Discussion of Study Design

Since the primary objective of the trial was to evaluate the safety of two new attenuated vaccine strains, an open-label planned dose escalation design was deemed appropriate.

Fecal excretion of vaccine and immunological parameters were evaluated by dose group. Vaccine was administered orally as a single dose on Day 0 to

eligible inpatient volunteers. Both vaccine strains were given in a dose-escalation design with escalation to the higher dose level dependent on lack of clinically significant effects at the lower dose level, the planned escalation was from 5×10^7 bacteria to 5×10^9 bacteria to 5×10^{10} bacteria.

6.3 Sel ction of Study Population

6.3.1 Inclusion Criteria

The following inclusion criteria were applied:

a. healthy, male or female inpatient volunteers, >18 or <50 years of age,
b. completed training on ETEC, diarrhea and protocol procedures,
c. demonstrate comprehension of the protocol procedures and knowledge of diarrhea, ETEC bacteria by passing a written examination, and
d. provide written informed consent.

6.3.2 Exclusion Criteria

The following exclusion criteria were applied:

a. chronic illness,
b. immunosuppressive condition,
c. positive serology for HbsAg, HCV, and/or HIV,
d. positive urine HCG within 4 days of immunization (women only),
e. antibiotics used within 7 days of vaccination,
f. significant abnormality in screening laboratory examinations (complete blood count with differential, blood chemistry, urinalysis),
g. if they travelled to a developing country within 5 years,
h. if they previously participated in an ETEC study,
i. regular use of laxatives or abnormal stool pattern, and
j. volunteers over the age of 40 with an abnormal EKG.

6.4 Removal of Subjects From Treatment or Assessment

Subjects were removed from the study if, in the opinion of the investigator, the health status of the subject warranted withdrawal (either through an adverse event or concurrent illness), there was significant non-compliance with the protocolled assessments or visits, or consent was withdrawn.

Where possible, follow-up assessments were conducted as protocolled, to the end of the appropriate treatment period (i.e. 14 days post vaccination) in all subjects who were withdrawn.

Subjects who were withdrawn from the study were not replaced.

6.4.1 Discontinuation of Treatment in a Specific Cohort of Subjects

The open-label study allowed both vaccine strains to be given in a dose-escalation design with escalation to the higher dose level dependent on

lack of clinically significant effects at the lower dose level, the planned escalation was from 5 x 10^7 bacteria to 5 x 10^9 bacteria to 5 x 10^{10} bacteria.

6.5 Treatm nts

6.5.1 Treatments Administered

For the three dose groups, on Day 0 stomach contents were buffered with 120ml sodium bicarbonate solution (1.33% w/v), immediately prior to the oral administration of either 5 x 10^7 bacteria, 5 x 10^9 bacteria, or 5 x 10^8 bacteria suspended in 30ml of sodium bicarbonate solution.

6.5.2 Identity of Investigational Product

The vials of seed lots of strains PTL-ETEC-002 and PTL-ETEC-003 (100 vials per lot of each strain) were supplied by Acambis Research Limited. (100, Fulbourn Rd., Cambridge CB1 9PT, United Kingdom) in clear, neutral type 1 glass vials sealed with grey butyl rubber stoppers.

Secondary seed lots (100 cryovials per lot of each strain; 1 mL of suspension in a 2 mL cryovial) were prepared at Johns Hopkins University (JHU), standard operating procedure for secondary seed lot preparation and related FDA correspondence is provided in **Appendix 12.7**. The JHU lot numbers were 071498 (PTL-ETEC-002) and 070898 (PTL-ETEC-003). The seeds were stored at –70°C in a temperature-monitored secure freezer in the laboratories of the VTU in the Johns Hopkins Bloomberg School of Public Health and then transported to the GCRC of the Johns Hopkins Hospital for administration to the volunteers. The Principal Investigator (PI), David Sack, M.D. and George Gomes made the vaccine preps. The actual vaccine dose was determined by plate counts performed on the inoculum after volunteer dosing was completed. This was performed in the VTU in the Johns Hopkins Bloomberg School of Public Health. After dosing, all vaccine remaining on that day was inactivated by autoclaving.

Buffer solution comprised a 1.33% w/v solution of sodium bicarbonate in water for injection.

6.6 Method of Assigning Subjects to Treatment Groups

Eligible subjects in the groups were sequentially assigned as they were screened to one of the two strains of bacteria.

6.7 Selection of Doses in the Study

The dose ranges of 5 x 10^7 to 5 x 10^{10} were chosen for this study. The parent strain of PTL-ETEC-002 and PTL-ETEC-003, E1392/75/2A was previously tested by Levine et al. (reviewed by Tacket, C. O. and M. M. Levine 1997)[8] as a live ETEC vaccine in two studies with doses ranging from 1 x 10^9 to 6 x 10^{10} bacteria. Diarrhea was seen as a side effect in approximately 10% of

volunteers receiving doses greater than 1×10^{10} bacteria. Because our strains are further attenuated we felt it was safe to escalate to 5×10^{10} bacteria.

6.8 Selection and Timing of Dose for Each Subject

As described in **Section 7.1**, subjects were sequentially assigned into one of two strains enrolled into the first dose group. Each group was assessed for clinical significant effects before proceeding to the next dose group. Each comprised the oral administration of 120ml of sodium bicarbonate solution to neutralize the gastric acid (which would otherwise diminish the potency of the vaccine), followed immediately by 30ml of vaccine bacteria suspended in buffer.

Volunteers were requested to fast for 90 minutes before and after administration of vaccine and were observed to ensure consumption of the entire contents of each vaccine.

6.9 Blinding

This was an open-label study and therefore no blinding mechanisms required or implemented.

6.10 Prior and Concomitant Therapy

Prior ETEC vaccination at any time, or treatment with antibiotics within 7 days of vaccination were grounds for exclusion or dismissal from the study. Anti-pyretics were not permitted during the follow-up period unless discussed beforehand with study personnel. This was to avoid masking of any vaccine-induced fever.

6.11 Treatment Compliance

All vaccinations were conducted in the inpatient unit at GCRC at the Johns Hopkins Hospital. A member of the VTU staff witnessed that the buffer and vaccine solutions were completely consumed, and documented on the vaccine accountability record.

6.12 Efficacy and Safety Variables

6.12.1 Efficacy and Safety Assessments

A direct assessment of efficacy (i.e. protection against ETEC) was not made or planned in this trial.

Excretion of the vaccine strains

Up to two stool specimens were collected each day after the immunization and were cultured on MacConkey agar and on MacConkey agar with streptomycin (Mac-strep). Colonies that grew on the Mac-strep plate were presumed to be vaccine strains and up to ten colonies were spotted onto Luria agar, a complete growth medium, and onto minimal media. Control (wild type) strains of *E. coli* grow on both of these agars, but the vaccine strains do not grow on the minimal media.

At least one colony of the vaccine strains was saved on nutrient agar slants.

Antibody lymphocyte supernatant (ALS) assays

Peripheral blood mononuclear lymphocytes (PBMLs) collected on the day of immunization and on days 7 and 10 post immunization were analyzed by antibody lymphocyte supernatant assay (ALS). Unstimulated PBMLs were cultured for 48 hours and the culture supernatant subjected to ELISA assay for CFA/II specific IgG or IgA similar to assay for serum antibodies (see following section).

Serum antibody assays

Serum specimens were obtained on day of immunization and on days 7 and 10 after the immunization, an addition serum sample was collected 14 days after immunization.. The serum specimens were assayed by ELISA for IgG antibodies to the CFA/II antigen using antigen provided by Acambis Research Limited. The assay was performed by pre-coating the plate with CFA/II antigen using a concentration of 1 μg/mL in PBS. After an overnight incubation at room temperature, the plates were blocked with BSA and washed. Three-fold dilutions of the volunteers' sera were prepared starting with a dilution of 1:10 in the first cup. The plates were incubated for one hour and subsequent to incubation with HRP-labeled anti-human IgG and substrate. Between each step, the plates were washed with PBS-Tween 20. The plates were read in an automatic ELISA reader where the wavelength was 450 mm.

To establish the appropriate concentration of CFA antigen for the assay, a validation study was carried out using varying dilutions of antigen, and sera from mice. Serum #1 was from mice that had been immunized with a CFA/II bearing strain. Serum #2 was from mice immunized with an isogenic strain without CFA/II expression, and serum #3 was from mice that had not been immunized. Using these reagents, the titration results were similar when the concentration of the CFA/II varied from 5 to 45 μg/mL. There was a slight drop in Absorbance values when the antigen concentration was lowered to 1 μg, and a major drop when the concentration was lowered to 0.2 μg. The titers of the two immunized mice were higher than the serum from the non-immunized mice, but the serum from the mice immunized with CFA-negative *E. coli* was significantly higher than the non-immunized mouse serum, suggesting that the CFA antigen contained some antigens from the bacteria in addition to CFA antigen. The concentration of 1 μg/mL appeared to be optimal for differentiating the two immune mice sera.

A standard serum pool was developed as a positive control. To make the standard, 0.3 mL of sera collected on day 10 from those volunteers who had received the dose of 10^9 CFU were pooled. The test serum from each volunteer was tested on the same plate on the same day and a titration of the standard serum was included on each plate.

Safety

Safety was assessed by way of investigator assessment twice daily (once shortly after vaccination), vital signs were taken three times a day

and fluid intake and output were measured on Days 0 through 6 post vaccination.

All stools were examined, graded and weighed by a nurse. The first two stools each day were sampled for microbiological examination and tested for occult blood. The stool consistency was graded as 1=formed, 2=soft/mushy, 3=thick liquid, 4=opaque watery, 5=rice in water.

Diarrhea was defined as two or more loose stools ($\geq$ grade 3 stools) in a period of 24 hours totalling 200 grams, or the occurrence of a single loose stool with a weight of 300 grams or more.

Dysentery was defined as the occurrence of diarrhea with blood in the stool as detected as grossly visible blood.

A fever was defined as the occurrence of an oral temperature $\geq$38.0◦C sustained in at least two occasions four hours apart. Where oral temperatures of $\geq$38.0◦C were recorded on two occasions four hours apart prior to Day 4, appropriate cultures were obtained and ciprofloxacin (500mg BID) was prescribed. If no symptoms developed following vaccination, the volunteers were given ciprofloxacin (500mg BID) for 3 days beginning on Day 4.

For the assessment of reactogenicity, all signs and symptoms of grade 1 or more were reviewed. Signs assessed included: ill appearance, rash, abdominal tenderness, liver palpable or spleen palpable. The signs were assessed as "Yes=present" or "No=not present". Symptoms assessed included: feels ill, poor appetite, nausea, vomiting, abdominal gurgling, gas, abdominal cramps, diarrhea, tenesmus, chills, malaise, bedridden, headache, lightheaded, and muscle aches. The symptoms were graded as 0=none, 1=mild; elicited on questioning, 2=moderate; self reported, 3=severe; symptoms interfere with normal function.

A serious adverse event (SAE) was defined as any untoward medical occurrence that at any dose: resulted in death, was life-threatening, required or prolonged hospitalization, resulted in persistent or significant disability/incapacity, or was a congential abnormality/birth defect. It was standard operating procedure of the GCRC and VTU to provide preliminary information on the SAE to the Principal Investigator who reported the SAE to the FDA, JCCI IRB and Acambis Research Limited within 24 hours of the knowledge of such an event.

Additional safety assessments included the determination of the extent and duration of bacterial shedding, by the collection of stool samples on Days 1-6 after vaccination and bacteriological assessment as described in 6.12.1.

At the discharge visit from the inpatient facility (Day 6) subjects were asked to contact the VTU if any signs and symptoms occurred in the succeeding 8 days.

6.13 Appropriateness of Measurements

Other clinical studies with ETEC vaccines have indicated that serum and intestinal antibodies to CFA II antigens are appropriate measures of responses to vaccination.

Because of the inpatient design of this study, the daily collection and evaluation of samples of all passed stools was feasible.

For documentation of safety, daily assessments of signs and symptoms, and vital signs in the inpatient facility was a consistent method of data collection and evaluation.

6.14 Drug Concentration Measurements

The measurement of circulating drug levels for a vaccine is considered inappropriate, since antibody titres and their duration are the primary measure of vaccine efficacy, both of which are unrelated to systemic drug concentration. Consequently systemic drug levels were not measured.

6.15 Data Management and Quality Assurance

All hematology, clinical chemistry and urinalysis samples were analyzed by a quality assurance accredited laboratory (certificate of accreditation is given in **Appendix 12.6**). No specific quality assurance systems were applied to the immunological assays, which were conducted at the VTU.

Source document verification as applicable to completion of case report forms was not carried out for this Investigator IND Study.

All clinical data i.e., all data with the exception of the bacteriological and immunological assays, were obtained by GCRC and VTU personnel and reside as hard copies in the subjects' records. The bacteriological and immunological data reside in notebooks and spreadsheets kept at the VTU.

6.16 Statistical Methods Planned in Protocol and Determination of Sample Size

6.16.1 Statistical and Analytical Plan

There was no formal statistical analysis plan for this Phase I trial.

Safety was evaluated by reviewing the completed case report forms and laboratory values for individual subjects by treatment group.

For each immunogenicity endpoint, the null hypothesis for all immunogenicity comparisons was that the immune response was the same for the two vaccine strains and across dose levels.

CFA/II antigen specific peripheral blood antibody secreting cells and serum and intestinal antibodies to CFA II antigens are appropriate measures of responses to vaccination.

For documentation of safety, the assessments of signs and symptoms via the diary card, and additional assessments in the outpatient facility was a consistent method of data collection and evaluation.

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All clinical data i.e., all data with the exception of the bacteriological and immunological assays, were obtained by VTU personnel and reside as hard copies in the subjects' records. The bacteriological and immunological data reside in notebooks and spreadsheets kept at the VTU and at NMRC.

6.16 Statistical Methods Planned in Protocol and Determination of Sample Size

6.16.1 Statistical and Analytical Plan

There was no formal statistical analysis plan for this Phase I outpatient trial. Safety was evaluated by reviewing the completed case report forms and laboratory values for individual subjects by treatment group. Adverse events were summarized by frequency of occurrence, number of subjects experiencing adverse events, severity and relationship to investigational vaccine. The frequency of occurrence of adverse events was compared by Fisher's Exact test between placebo and vaccine recipients.

Immune response to the vaccine was determined qualitatively, the criteria for determining positive and negative responders were determined prior to unblinding and the assignment of positive and negative responders was also performed on blinded data.

6.16.2 Determination of Sample Size

The number of subjects planned for the study was based on logistical considerations rather than power calculations.

6.17 Protocol Amendments

No formal protocol amendments were made during the conduct of this outpatient protocol.

7 STUDY SUBJECTS

7.1 Disposition of Subjects

Forty-two (42) subjects were enrolled in the outpatient phase of the study. Two individuals withdrew before receiving vaccine. The remaining 40 subjects were randomly assigned to one of six groups to receive vaccine or placebo on Days 0 and 10 as shown in **Table 1**. Twelve were assigned to receive two doses of PTL-ETEC-002 and 12 to receive two doses of PTL-ETEC-003. Four (4) were randomized into each of the single-dose groups to receive vaccine followed by placebo or vice versa. All volunteers received the first dose of vaccine or placebo and 37 received the second dose. The three (3) who missed the second dose were in the groups assigned to receive PTL-ETEC-002/PTL-ETEC-002, PTL-ETEC-003/PTL-ETEC-003, and PTL-ETEC-002/placebo. These missed doses resulted in administration of vaccine/placebo as shown in **Table 2**.

Table 1
Number of Subjects Planned and Analyzed

# Subjects Planned	# Subjects Screened	# Subjects Completed	# Subjects Withdrew
40	42	40	2

Table 2
Allocation of Treatment to Outpatient Volunteers

Group	Number of Subjects	Day 0	Day 10
1	11	PTL-ETEC-002	PTL-ETEC-002
2	11	PTL-ETEC-003	PTL-ETEC-003
3	4	placebo	PTL-ETEC-002
4	4	placebo	PTL-ETEC-003
5	4	PTL-ETEC-002	placebo
6	4	PTL-ETEC-003	placebo

Key demographic variables of age, sex, race and treatment group are summarized in **Table 3.** Of the 40 healthy subjects, 10 were male and 30 were female; 7 were african-american, 28 were caucasian, and 5 were other, with an age range from 18 to 50 years.

Table 3
Demographics

Variable[1]	Treatment Group					
	PTL-ETEC-002			PTL-ETEC-003		
	2 Doses (Group 1)	2nd Dose (Group 3)	1st Dose (Group 5)	2 Doses (Group 2)	2nd Dose (Group 4)	1st Dose (Group 6)
Age (Yrs.)						
Mean	28	35	27	35	32	38
S. D.	5	8	5	7	6	8
Range	21-37	22-50	22-35	22-48	22-43	29-50
Gender						
Male	2	1	2	4	0	1
Female	9	3	3	7	4	4
Ethnicity						
Caucasian	9	3	5	7	3	1
African-American	1	1	0	2	1	2
Asian	0	0	0	0	0	0
Other	1	0	0	2	0	2

[1] Demographics determined at the time of vaccination.

7.2 Protocol Deviations

No formal protocol deviations were documented. In total 40 volunteers received the first dose of vaccine or placebo and 37 received the second dose. The three (3) who missed the second dose were in the groups assigned to receive PTL-ETEC-002/PTL-ETEC-002, PTL-ETEC-003/PTL-ETEC-003, and PTL-ETEC-002/placebo as shown in **Table 2**. It was thought that these were sufficient numbers exposed to each strain to fulfill the objectives of the trial.

One patient assigned to the PTL-ETEC-002/PTL-ETEC-002 group dropped out of the study after receiving vaccine on Day 10. Since no samples were collected after the second dose, data from this subject are included with data of those who received one dose of PTL-ETEC-002. Data are, therefore, available for all 40 subjects following one dose of vaccine, for 10 following a second dose of PTL-ETEC-002, and for 11 following a second dose of PTL-ETEC-003.

It is noted that there are study related procedures that were not consistently documented on the case report forms and/or source documents as delineated in the protocol.

7.3 Extent of Exposure

The number of subjects who received treatment is shown in **Table 4**.

Table 4
Treatment Groups

Group	Number of Subjects	Day 0	Day 10
1	10	PTL-ETEC-002	PTL-ETEC-002
2	11	PTL-ETEC-003	PTL-ETEC-003
3	4	placebo	PTL-ETEC-002
4	4	placebo	PTL-ETEC-003
5	6	PTL-ETEC-002	placebo or not dose[1]
6	5	PTL-ETEC-003	placebo or not dose[2]

[1] 3 received placebo; 2 not dosed
[2] 4 received placebo; 1 not dosed

In all cases, the vaccine solution was fully consumed. There was only one reported case of vomiting which occurred on Day 7 after receipt of a placebo dose (Subject #36) and was, thus, not related to the study vaccine.

8 EFFICACY EVALUATION

8.1 Data Sets Analysed

All 40 of the volunteers provided blood samples at Day 0 and Day 7. One subject who received two doses of PTL-ETEC-002 did not return after the second vaccination. One assigned to receive PTL-ETEC-002/placebo missed the visit at Day 10, and one who received PTL-ETEC-003/placebo missed a single visit at Day 17. All available data from the 40 subjects who received one or two doses of study vaccine were reviewed and samples analyzed.

8.2 Efficacy/Immunogenicity Results

Specific antibody secreted by PBMCs

The antibody-secreting cell (ASC) response was determined in peripheral blood mononuclear lymphocytes (PBMLs) by comparing the number of ELISPOTS at baseline (Day 0) with results 7 days after vaccine administration (Days 7 or 17). An ASC response was defined as 1.3 or more spots per 10^6

cells 7 days after vaccination and, if the baseline value was positive, a doubling of the baseline value. PBMLs were also analyzed by antibody lymphocyte supernatant assay (ALS). Unstimulated PBMLs were cultured for 48 hours and the culture supernatant subjected to ELISA assay for CFA/II specific IgG or IgA. An ALS response was defined as a 2-fold or greater increase compared to the baseline sample.

All 40 of the volunteers provided blood samples at Day 0 and Day 7. As described above, one subject who received two doses of PTL-ETEC-002 did not return after the second vaccination. One assigned to receive PTL-ETEC-002/placebo missed the visit at Day 10, and one who received PTL-ETEC-003/placebo missed a single visit at Day 17. Therefore, PBMCs were available at baseline and 7 days after one dose of vaccine for all 40 subjects, 20 of who received PTL-ETEC-002 and 20, PTL-ETEC-003. In addition, PBMCs were available 7 days after a second dose of vaccine for 10 who received a second dose of PTL-ETEC-002 and 11 who received a second dose of PTL-ETEC-003. Results are shown in the following **Tables 5 - 8.**

Table 5
Number of Subjects with IgA anti-CFA/II ASC Response Following One or Two Doses of PTL-ETEC-002 or PTL-ETEC-003

Vaccine Responders	Responders after one dose	New responders[1] after second dose	Total responders after two doses	Total responders after one or two doses
PTL-ETEC-002	4/10 (40%)	1/5 (20%)	7/10 (70%)	11/20 (55%)
PTL-ETEC-003	9/9 (100%)	3/5 (60%)	9/11 (82%)	18/20 (90%)

[1]The number of subjects who responded to the 2nd dose but not the first dose/number who did not respond to the first dose

Table 6
Number of Subjects with IgG anti-CFA/II ASC Response Following One or Two Doses of PTL-ETEC-002 or PTL-ETEC-003

Vaccine Responders	Responders after one dose	New responders after second dose	Total responders after two doses	Total responders after one or two doses
PTL-ETEC-002	3/10 (30%)	0/7 (0%)	3/10 (30%)	6/20 (30%)
PTL-ETEC-003	5/9 (56%)	1/7 (14%)	5/10 (40%)	10/20 (50%)

Table 7
Number of Subjects with IgA anti-CFA/II ALS Response Following One or Two Doses of PTL-ETEC-002 or PTL-ETEC-003

Vaccine Responders	Responders after one dose	New responders after second dose	Total responders after two doses	Total responders after one or two doses
PTL-ETEC-002	9/20 (45%)	0/5 (0%)	5/10 (50%)	9/20 (45%)
PTL-ETEC-003	10/20 (50%)	4/7 (57%)	8/11 (73%)	14/20 (70%)

Table 8
Number of Subjects with IgG anti-CFA/II ALS Response Following One or Two Doses of PTL-ETEC-002 or PTL-ETEC-003

Vaccine Responders	Responders after one dose	New responders after second dose	Total responders after two doses	Total responders after one or two doses
PTL-ETEC-002	2/20 (10%)	1/9 (11%)	2/10 (20%)	3/20 (15%)
PTL-ETEC-003	6/20 (30%)	2/9 (22%)	4/11 (36%)	8/20 (40%)

Specific serum IgA and IgG

Blood for measurement of anti-CFA/II IgA and IgG in the serum was obtained on Days 0, 7, 10, 17, 24, and 38 (see **Tables 9** and **10**). A response was defined as a 2-fold or greater increase compared to the antibody level on Day 0. For those receiving two doses of vaccine, responses on Days 7 and 10 were attributed to the first dose and responses after Day 10 were attributed to the second dose of vaccine.

Table 9
Number of Subjects with Serum IgA anti-CFA/II Response Following One or Two Doses of PTL-ETEC-002 or PTL-ETEC-003

Vaccine Responders	Responders after one dose	New responders after second dose	Total responders after two doses	Total responders after one or two doses
PTL-ETEC-002	0/20 (0%)	0/20 (0%)	0/10 (0%)	0/20 (0%)
PTL-ETEC-003	5/9 (56%)	1/10 (10%)	2/11 (18%)	7/20 (35%)

Table 10
Number of Subjects with Serum IgG anti-CFA/II Response Following One or Two Doses of PTL-ETEC-002 or PTL-ETEC-003

Vaccine Responders	Responders after one dose	New responders after second dose	Total responders after two doses	Total responders after one or two doses
PTL-ETEC-002	1/10 (10%)	0/10 (0%)	3/10 (30%)	4/20 (20%)
PTL-ETEC-003	43/9 (44%)	0/10 (0%)	4/11 (36%)	8/20 (40%)

Specific Fecal IgA

Fecal samples for measurement of anti-CFA/II IgA was obtained on Days 0, 7, 10, 17 and 38 (see **Table 11**). Although all the samples were run by ELISA, evaluable sets of samples were subjected to the following criteria: 1) Total IgA concentration must be at least 20 μg/mL fecal extract, 2) The total IgA concentration of the pair being evaluation must not exceed a 10-fold difference. A response was defined as a 2-fold or greater increase compared to the antibody level on Day 0. For those receiving two doses of vaccine, responses on Days 7 and 10 were attributed to the first dose and responses after Day 10 were attributed to the second dose of vaccine.

Table 11
Number of Subjects with Fecal IgA anti-CFA/II Response Following One or Two Doses of PTL-ETEC-002 or PTL-ETEC-003

Vaccine Responders	Responders after one dose	New responders after second dose	Total responders after two doses	Total responders after one or two doses
PTL-ETEC-002	1/6 17%)	0/5 (0%)	1/6 (17%)	2/12 (17%)
PTL-ETEC-003	2/5 (40%)	2/9 (22%)	2/9 (22%)	4/14 (29%)

9 SAFETY EVALUATION

9.1 Extent of Exposure

All 40 subjects who received one or two doses of either the live vaccine strain PTL-ETEC-002 or PTL-ETEC 003 and/or placebo according to protocol were included in the analyses.

9.2 Bacteriology

9.2.1 Excretion of the Vaccine Strains

Colonization with either PTL-ETEC-002 or PTL-ETEC-003 ETEC strains was assessed by culture of stool specimens collected by the subject on Day 0, 3, 6-7, 10, 11-12, 16-18, 20-21, 24-25. For those subjects who continue to shed bacteria on Day 24-25 or beyond, specimens were collected and cultured until 2 negative stool cultures were obtained: on Day 33-36, 38, 41-43, and 53-54. For purposes of simplification, Day 6-7 will be referred to as Day 6, Day 11-12 as Day 11, Day 16-18 as Day 16, Day 20-21 as Day 20, Day 24-25 as Day 24, Day 33-36 as Day 33, Day 41-43 as Day 41, Day 53-54 as Day 53.

Stool specimens collected were cultured on MacConkey agar and on MacConkey agar with streptomycin. Colonies that grew on the Mac-strep plate were presumed to be vaccine strains and five colonies were spotted onto Luria agar and onto minimal media (Davies). Control (wild type) strains of *E. coli* grow on both of these agars, but the vaccine strains do not grow on the minimal media. At least one colony of the vaccine strains was saved on nutrient agar slants.

From a maximum of 280 protocolled stool samples/rectal swabs (scheduled as above), 117 (84%) were collected for culture. Nine (9) of 10 subjects receiving 2 doses of PTL-ETEC-002 had positive stool cultures after either 1 or 2 doses. Of the 10 subjects who only received a single dose of PTL-ETEC-002, either by design (i.e. received placebo as the other dose) or due to illness or drop-out, 8 subjects were stool culture positive on at least one occasion (**Table 12**).

Table 12
Duration and Time Course of Fecal Shedding of PTL-ETEC-002 in Individual Subjects

Vaccine	Volunteer #	Dose 1		Dose 2	
		# Positive stools	Days post vaccination	# Positive stools	Days post vaccination
PTL-002 one dose	31	NA[1]	-	0	-
	40	NA	-	9	6-14
	50	NA	-	2	1, 31
	54	NA	-	6	1-6
	33	14	11-24	NA	-
	38	10	3-6, 11-16	NA	-
	62	1	3	NA	-
	69	1[2]	6	NA	-
	59	1	1	NA	-
PTL-002 two Doses	30	1	6	0	-
	41	0	-	1	1
	42	0	-	2	0-1
	44	0	-	0[3]	-
	46	1	6	1	1
	48	0	-	1	1
	52	1	3	6	1-6
	53	1	3	1	1
	60	1	3	0	-
	64	0	-	0	-
	71	0	-	1	1

1 NA=not applicable, no active vaccine administered.
2 No samples were collected on Days 10 and 11 and continued fecal shedding after Day 6 cannot be determined. Samples beyond Day 16 were culture negative.
3 This subject (#44) received the second dose of vaccine on Day 10, had a culture negative stool on Day 11-12 and dropped out of the study. No subsequent samples were collected.

All 11 subjects receiving 2 doses of PTL-ETEC-003 were stool culture positive after either one or 2 doses of vaccine. Eight of the 9 subjects receiving a single dose of PTL-ETEC-003, either by design or due to illness or drop-out, had positive stool cultures (**Table 13**).

Table 13
Duration and Time Course of Fecal Shedding of PTL-ETEC-003 in Individual Subjects

Vaccine	Volunteer #	Dose 1		Dose 2	
		# Positive stools	Days post vaccination	# Positive stools	Days post vaccination
PTL-003 one dose	32	NA	-	14	1-14
	39	NA	-	14	1-14
	66	NA	-	10	1-10
	68	NA	-	0	-
	34	8	3-10	NA	-
	37	19	6-24	NA	-
	57	9	3-11	NA	-
	65	9	3-10, 20	NA	-
	45	10	3-11, 24	NA	-
PTL-003 two doses	35	0	-	7	0-6
	36	1	3	1	1
	43	1	6	2	0-1
	47	0	-	1	1

Table 13
Duration and Time Course of Fecal Shedding of PTL-ETEC-003 in Individual Subjects

Vaccine	Volunteer #	Dose 1		Dose 2	
		# Positive stools	Days post vaccination	# Positive stools	Days post vaccination
	49	1	6	1	1
	51	1	3	1	0
	55	8	3-10	2[1]	0-1
	56	0	-	1	1
	58	1	3	0	-
	67	1	3	14	1-14
	70	3	1-3	1[2]	1

PTL-003 = PTL-ETEC-003
[1]Continued shedding on Day 10 and 11 after dose 1 maybe attributable to dose 1 and not dose 2.
[2]No stool samples were collected for the 2 time points flanking Day 1 after second dose.

Duration of fecal shedding of ETEC

The number of positive stool samples per subject receiving PTL-ETEC-002 ranged from 0 - 14 with a median of 1 and an average of 2.1 (**Table 12**). The number of positive stool samples per subject receiving PTL-ETEC-003 ranged from 0 - 19 with a median of 1 and an average of 4.6 (**Table 12**). The number of subjects with positive stool samples after vaccination with PTL-ETEC-002 or PTL-ETEC-003 is shown in **Figure 1**. The data indicate that the majority of recipients had either no or one positive stools. It should be noted that since subjects receiving 2 doses of vaccine are counted twice, once after the first dose and then again after the second dose, the majority of cases (6 of 9 for PTL-ETEC-002 and 3 of 4 for PTL-ETEC-003) with no positive stools occurred after receiving the first dose of active vaccine and the subjects were culture positive following the second dose of active vaccine (**Table 12**).

Figure 1

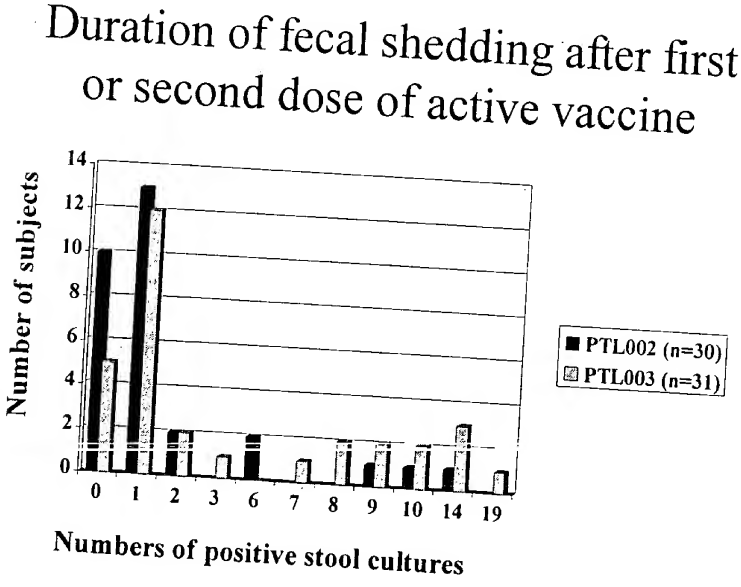

Figure 1. The number of culture positive stool samples from each subject following each dose of active vaccine is shown. Subjects receiving 2 doses of active vaccine are counted twice with all bacterial shedding following the second dose of vaccine attributed to Dose 2, and not Dose 1.

Time course of fecal shedding of vaccine strains following vaccination

The number of subjects with positive stool cultures is plotted against Day post vaccination to give an indication of the time course of fecal shedding of vaccine strains (**Figures 2 and 3**).

Figure 2

Time course of fecal shedding in subjects receiving one dose of active vaccine

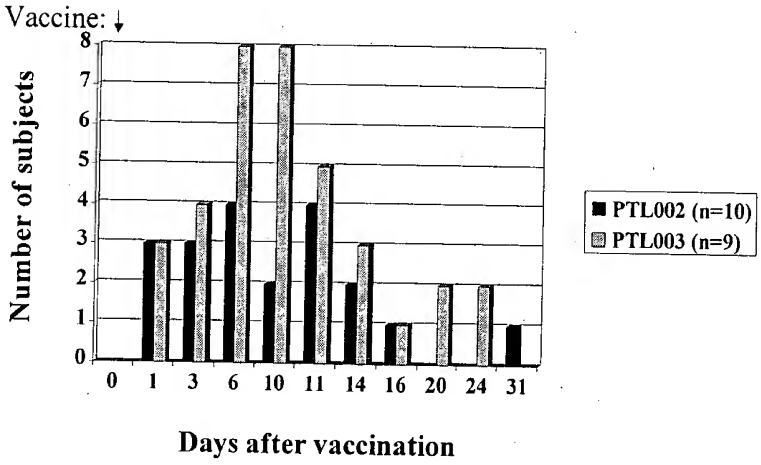

Figure 2. Subjects received a single dose of vaccine on Day 0. For subjects who received active vaccine on Dose 2 schedule (i. e., Dose 1 was placebo), 10 days were subtracted from the Study Day to derive the correct # days post vaccination. The number of subjects with positive stool cultures for the specified post-vaccination day is shown.

Figure 3

Time course of fecal shedding in subjects receiving 2 doses of active vaccine

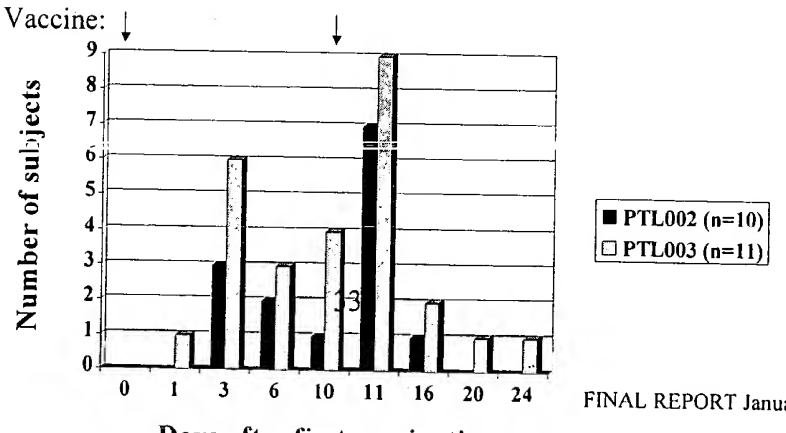

Figure 3. Subjects received 2 doses of vaccine on Day 0 and Day 10. The number of subjects with positive stool cultures for the specified post - first vaccination is shown.

Data obtained from subjects receiving a single dose of active vaccine, by design or due to illness or drop-out, indicate that shedding of strain PTL-ETEC-002 commenced on Day 1 but can occur as long as 31 days post vaccination, with the majority of shedding occurring between 1 and 11 days post vaccination (**Figure 2**). Shedding of strain PTL-ETEC-003 occurs between 1 and 24 days post vaccination with the majority of shedding between days 1 - 14 and peaking 6 - 10 days post vaccination (**Figure 2**).

The data obtained from subjects receiving 2 doses of vaccine are more difficult to interpret as fecal shedding after Day 10 can be attributable to either Dose 1 or Dose 2 (**Figure 3**). Receipt of 2 doses of vaccine does not appear to prolong the period of shedding compared with the subjects shown in **Figure 2** who received only one dose. No positive stools occurred beyond day 16 for PTL-ETEC-002 and day 24 for PTL-ETEC-003 (**Figure 3**). The number of positive stools reached a peak 11 days post vaccination but many of these are probably a result of the second vaccination since most of the subjects were culture negative during the days prior to receipt of the second dose (**Figure 3**).

9.3 Reactogenicity: Symptoms and Adverse Events

9.3.1 Summary of symptoms and adverse events

The number and percent of incidences of subjects experiencing symptoms captured from diary cards are tallied for each of the three treatment groups (**Table 14**). The numbers shown are incidences per subject per dose; a subject may be counted twice if he/she experienced symptoms after both doses. All incidences occurring from Day 0 to Day 7 after dosing are included. Several incidences of symptoms with onsets on Day -1 of dosing were not included in this analysis. There were 2 subjects who missed their second dose of either PTL-ETEC-002 or PTL-ETEC-003 that were counted towards the placebo group and follow-up samples and adverse events were collected at the prescribed times. There was one drop-out that did not receive a second dose of PTL-ETEC-002 and was not allocated to the placebo group since there was no follow-up information.

The overall incidences of general symptoms were not significantly different (Fisher's exact test) for subjects receiving either PTL-ETEC-002 or PTL-ETEC-003, compared to placebo recipients.

There were no incidences of oral temperature greater that 100.4 °F. There were reports of illness, weakness, headache, light-headedness, muscle aches and chills but the percent of subjects in any one group experiencing these symptoms never exceeded 20%. Headache was the most common complaint with incidences of 17% - 19% after receiving placebo, PTL-ETEC-002, or PTL-ETEC-003.

Table 14
Incidence of All Symptoms Recorded on Diary Cards from Day 0 to Day 7 After Each Dose

Symptom	Number (% Incidence)			P Value (Fisher's Exact Test)	
	Placebo N=18[1]	PTL-002[2] N=30[3]	PTL-003[4] N=31[5]	Placebo vs. PTL-ETEC-002	Placebo vs. PTL-ETEC-003
General:					
Oral Temperature (>100.4°F)	0 (0%)	0 (0%)	0 (0%)	1.00	1.00
Felt Ill	0 (0%)	3 (10%)	2 (7%)	0.28	0.53
Weakness	1 (6%)	2 (7%)	1 (3%)	1.00	1.00
Headache	3 (17%)	5 (17%)	6 (19%)	1.00	1.00
Lightheadedness	0 (0%)	3 (10%)	3 (10%)	0.28	0.29
Muscle Aches	1 (6%)	1 (3%)	0 (0%)	1.00	0.37
Chills	0 (0%)	0 (0%)	2 (7%)	0.38	1.00
Gastrointestinal:					
Decreased Appetite	0 (0%)	0 (0%)	1 (3%)	1.00	1.00
Gas	3 (17%)	8 (27%)	4 (13%)	0.50	0.70
Cramping	3 (17%)	7 (23%)	2 (7%)	0.72	0.34
Nausea	0 (0%)	1 (3%)	1 (3%)	1.00	1.00
Abdominal Pain	0 (0%)	1 (3%)	2 (7%)	1.00	0.53
Gurgling Stomach	1 (6%)	3 (10%)	1 (3%)	1.00	1.00
Blood in Stool	0 (0%)	0 (0%)	0 (0%)	1.00	1.00
Belching	0 (0%)	0 (0%)	0 (0%)	1.00	1.00
Urgency of Defecation	1 (6%)	4 (13%)	1 (3%)	0.64	1.00
Pain in the Rectum with Defecation	1 (6%)	1 (3%)	1 (3%)	1.00	1.00
Vomiting	1 (6%)	0 (0%)	0 (0%)	1.00	1.00
Unformed Stools	4 (22%)	3 (10%)	4 (13%)	0.40	0.44
Other	0 (0%)	0 (0%)	0 (0%)	1.00	1.00

[1] Of the 16 planned placebo doses, 2 subjects that did not receive their second dose of PTL-ETEC-002 or PTL-ETEC-003 vaccine were re-allocated to placebo treatment since follow-up samples and information were collected.
[2] PTL-002=PTL-ETEC-002
[3] Of the planned 32 PTL-ETEC-002 doses, one was lost to follow-up after the first dose and a second did not receive the second dose and was re-allocated to placebo treatment.
[4] PTL-003=PTL-ETEC-003
[5] Of the planned 32 PTL-ETEC-003 doses, one did not receive the second dose and was re-allocated to placebo treatment.

Gastrointestinal symptoms associated with infections with wild type ETEC were not found more frequently in subjects receiving PTL-ETEC-002 or

PTL-ETEC-003 compared with those receiving placebo or untreated (**Table 14**). There were no reports of blood in stool or belching. There were less than 5 reported cases in the combined groups of decreased appetite, nausea, abdominal pain, gurgling stomach, pain in the rectum with defecation and vomiting. There were 15 reports of gas, 12 reports of cramping, 11 reports of unformed stool, and 6 reports of urgency of defecation. None of the incidences were statistically different after receipt of PTL-ETEC-002 or PTL-ETEC-003 compared to placebo or no treatment (Fisher's exact test). Only two episodes of diarrhea (>3 unformed or liquid stool during a 24 hr period) and one episode of vomiting were detected during the trial. The diarrhea occurred in 1 vaccine (PTL-ETEC-003) recipient and 1 placebo recipient, and the vomiting occurred in a placebo recipient.

The above incidences of symptoms were further analyzed by comparison of incidences that occurred followed one or two doses of the same vaccine and segregated by severity. By design, there were no subjects receiving two doses of placebo and only those receiving the two consecutive doses of either PTL-ETEC-002 or PTL-ETEC-003 were included in the tally for second dose recipients (**Table 15**).

Table 15
Incidences of Symptoms Occurring within 7 Days After Either One or Two Doses

Characteristics	Number of Recipients with Symptoms/ Total Number of Recipients (%)			Fisher's exact test (p value) 1 vs. 2 doses	
	Placebo	PTL-002[1]	PTL-003[2]	PTL-002[1]	PTL-003[2]
Any symptom					
Any symptom of any severity within 7 days after first dose	8/18 (44%)	9/20 (45%)	8/20 (40%)	0.26	0.48
Any symptom of any severity within 7 days after second dose	N/A[3]	7/10 (70%)	6/11 (55%)	N/A	N/A
Any moderate or severe symptom within 7 days of first dose	1/18 (6%)	0/20 (0%)	2/20 (10%)	1.00	0.53
Any moderate or severe symptom within 7 days of second dose	N/A	0/10 (0%)	0/11 (0%)	N/A	N/A
Vomiting					
Vomiting within 7 days of first dose	0/18 (0%)	0/20 (0%)	0/20 (0%)	1.00	0.36
Vomiting within 7 days of second dose	1/18 (6%)	0/10 (0%)	0/11 (0%)	N/A	N/A
Loose stools					
Any loose stool within 7 days of first dose	4/18 (22%)	2/20 (10%)	3/20 (15%)	1.00	1.00
Any loose stool within 7 days of second dose	N/A	1/10 (10%)	1/11 (9%)	N/A	N/A
Any liquid or 'rice in water' stool within 7 days of first dose	1/18 (6%)	1/20 (5%)	1/20 (5%)	1.00	1.00

Table 15
Incidences of Symptoms Occurring within 7 Days After Either One or Two Doses

Characteristics	Number of Recipients with Symptoms/ Total Number of Recipients (%)			Fisher's exact test (p value) 1 vs. 2 doses	
	Placebo	PTL-002[1]	PTL-003[2]	PTL-002[1]	PTL-003[2]
Any liquid or 'rice in water' stool within 7 days of second dose	N/A	0/10 (0%)	1/11 (9%)	N/A	N/A
3 or more loose stools in a 24 hour period within 7 days of first dose	0/18 (0%)	0/20 (0%)	0/20 (0%)	1.00	1.00
3 or more loose stools in a 24 hour period within 7 days of second dose	1/18 (6%)	0/10 (0%)	1/11 (9%)	N/A	N/A
Any loose stool with moderate or severe intensity within 7 days of first dose	1/18 (6%)	0/20 (0%)	1/20 (5%)	1.00	1.00
Any loose stool with moderate or severe intensity within 7 days of second dose	N/A	0/10 (0%)	0/11 (0%)	N/A	N/A
Fever					
Fever (>100.4 °F) within 7 days of first dose	0/18 (0%)	0/20 (0%)	0/20 (0%)	1.00	1.00
Fever (>100.4 °F) within 7 days of second dose	N/A	0/10 (0%)	0/11 (0%)	N/A	N/A

1 PTL-002=PTL-ETEC-002
2 PTL-003=PTL-ETEC-003
3 N/A=Not Applicable

The data summarized in **Table 15** show that the incidences of symptoms were neither more nor less frequent after two doses in comparison with one dose.

A listing of all symptoms rated moderate or severe intensities is shown in **Table 16**.

Table 16
Incidences of All Symptoms Rated Moderate or Severe Intensities

Subject Identification	Treatment Related to Episode	Symptom	Intensity	Duration of Episode[3]
65	Placebo	Unformed stools	Moderate	Days -1, 0, 5, 6
48	PTL-002[1]	Felt Ill	Moderate	Days 3, 4, 5
		Light-headedness	Moderate	Day 4
		Weakness	Moderate	Days 3, 4, 5
37	PTL-003[2]	Light-headedness	Severe	Day 7
65	PTL-003[2]	Unformed stools	Moderate	Days 4, 6, 7
70	PTL-003[2]	Abdominal pain	Moderate	Days 0, 1, 2
		Felt Ill	Moderate	Days 0, 1, 2

1 PTL-002=PTL-ETEC-002
2 PTL-003=PTL-ETEC-003
3 Days with reports of moderate to severe symptoms shown. Days with reports of mild symptoms not included.

There were 5 episodes of symptoms with moderate or severe intensities and in

2 of these episodes, 2 or more symptoms had moderate intensities (**Table 16**).

- Subject 65 reported unformed stools throughout the study and the episodes are not likely to be treatment related.
- Subject 48 reported a 'head cold' on Day 1 after receipt of PTL-ETEC-002 and the subsequent symptoms of moderate intensity are probably attributable to this illness.
- Subject 37 reported severe light-headedness 7 days following receipt of PTL-ETEC-003, the delayed onset suggest that this episode is not related to receipt of vaccine.
- Subject 70 reported decreased appetite (level 1) one day prior to receipt of PTL-ETEC-003, this progressed to abdominal pain of moderate severity on Day 0, headache (level 1) was also reported starting on Day 0, as were feelings of illness with onset at Day 0 at level 1 and progressing to moderate intensity by Day 1. All symptoms were resolved by Day 4. It is likely that subject #70 contracted an unrelated illness.

9.3.2 Fever

A fever was defined as the occurrence of an oral temperature >38.0°C sustained in at least two occasions four hours apart. Where oral temperatures of ≥38.0°C were recorded on two occasions four hours apart prior to Day 4, appropriate cultures were obtained and ciprofloxacin (500mg BID for 3 days) was prescribed. If the vaccine strain was still present in the stool 14 days after the second dose, ciprofloxacin was to be administered (500 mg BID) and at least two follow-up stool specimens were to be collected to verify that the vaccine strain has been cleared.

There were no incidences of fever where oral temperatures exceeded 100.4 °F (**Table 15**).

9.3.3 Diarrhea or Loose Stools

All stools were examined, graded and weighed by the nurse. The first two stools each day were to be sampled for microbiological examination and tested for occult blood. The stool consistency was graded as 1=formed, 2=soft/mushy, 3=thick liquid, 4=opaque watery, 5=rice in water.

Diarrhea was defined as the passage of >3 unformed or liquid stools in a 24 hr period.

Dysentery was defined as the occurrence of diarrhea with blood in the stool as detected as grossly visible blood.

The incidence of unformed stools were neither more frequent following receipt of PTL-ETEC-002 or PTL-ETEC-003 (10% and 13%) compared to placebo or no treatment (22%; **Table 14**) nor less frequent following receipt of a second dose of PTL-ETEC-002 or PTL-ETEC-003 (10 and 15% after dose 1 compared to 10 and 9% after dose 2, **Table 15**).

Unformed stools were further defined by 1) descriptive terms: thick liquid, liquid, or rice in water diarrhea, 2) number of stools per 24 hour period, and 3) intensity: mild, moderate, or severe. When incidences of liquid or rice in water diarrhea were compared between treatments, no statistically significant differences were detected (**Table 15**). Similarly, incidences of 3 or more loose stools in a 24 hour period were comparable across treatment groups (**Table 15**). There were only 2 incidences of loose stool with moderate intensity and none with severe intensity. Both episodes of moderate intensities occurred in the same subject (Subject #65; **Table 16**), the first episode following receipt of PTL-ETEC-003 and the second following receipt of placebo (**Table 15**).

The time of onset and duration of incidences of unformed stools relative to the vaccine dose is shown in **Table 17**. There is no trend in either onset or duration that may suggest a causal relationship of unformed stools with receipt of PTL-ETEC-002 or PTL-ETEC-003.

The data in **Table 17** show that of the 11 episodes of unformed stools, 8 episodes had times of onset suggesting that they are unrelated to receipt of vaccine or placebo. Five episodes had onsets prior to receipt of vaccine and another 3 had onsets on day 5 or 7. Of the 3 episodes that occurred on day 0 or 1, one of the subjects (#65) appeared to have persistent intermittent loose stools throughout the study before or after receipt of placebo or PTL-ETEC-003. The remaining 2 episodes had day 1 onsets after receipt of PTL-ETEC-002 (Subject #33) or PTL-ETEC-003 (Subject #35) both episodes lasted only one day with either 1 or 3 unformed stools.

Only two episodes of diarrhea occurred during the trial period. These episodes occurred in 1 vaccine recipient (PTL-ETEC-003) and in 1 placebo recipient.

Table 17
Time of Onset, Duration and Intensity of Unformed Stool Episodes

Subject Identification	Treatment Related to Episode	Onset (Days Post Vaccination)	Duration (Days)	Number of Episodes
40	Placebo	7	1	2
57	Placebo	5	2	2
65	No treatment	-1	7 (intermittent)	7
30	PTL-002[1] dose 1	-1	4 (intermittent)	4
30	PTL-002[1] dose 2	-1	5 (intermittent)	5
33	PTL-002[1]	1	1	1
32	PTL-003[2]	-1	2 (intermittent)	2
35	PTL-003[2]	1	1	3
36	PTL-003[2]	5	1	1
37	PTL-003[2]	-1	1	4
65	PTL-003[2]	0	8	11

1 PTL-002=PTL-ETEC-002
2 PTL-003=PTL-ETEC-003

9.3.4 Vomiting

There was only one reported case of vomiting (volunteer #37) which occurred on Day 7 after receipt of placebo. Thus, this was not related to the vaccines under evaluation.

9.3.5 Deaths and Other Serious Adverse Events

There were no deaths or serious adverse events reported during the course of this protocol.

9.3.6 Clinical Laboratory Evaluation

There were no clinical laboratory data evaluated for this protocol.

9.3.7 Safety Conclusions

Two live oral vaccine strains PTL-ETEC-002 and PTL-ETEC-003 were given to outpatient volunteers at the Vaccine Testing Unit at Johns Hopkins University Bloomberg School of Public Health. All volunteers received the first dose of vaccine or placebo and 37 received the second dose. They were given oral doses of $2x10^9$ CFU using 200 ml of CeraVacx™ (a rice-based buffer containing 2 grams of sodium bicarbonate, 0.5 grams of trisodium citrate in addition to 7 grams of a proprietary rice syrup) containing the vaccine. The data available for all 40 subjects following one dose of vaccine, for 10 subjects following a second dose of PTL-ETEC-002, and for 11 subjects following a second dose of PTL-ETEC-003.

The overall incidences of general symptoms were not significantly different (Fisher's exact test) for subjects receiving either PTL-ETEC-002 or PTL-ETEC-003, compared to placebo recipients. Headache was the most common complaint with incidences of 17% - 19% after receiving placebo, PTL-ETEC-002, or PTL-ETEC-003. In addition, the incidences of symptoms were neither more nor less frequent after two doses in comparison with one dose.

Gastrointestinal symptoms associated with infections with wild type ETEC were not found more frequently in subjects receiving PTL-ETEC-002 or PTL-ETEC-003 compared with those receiving placebo or untreated. There were no reports of blood in stool or belching. There were less than 5 reported cases in the combined groups of decreased appetite, nausea, abdominal pain, gurgling stomach, pain in the rectum with defecation and vomiting. There were 15 reports of gas, 12 reports of cramping, 11 reports of unformed stool, and 6 reports of urgency of defecation. None of the incidences were statistically different after receipt of PTL-ETEC-002 or PTL-ETEC-003 compared to placebo or no treatment (Fisher's exact test). Only two episodes of diarrhea (>3 unformed or liquid stool during a 24 hr period) and one episode of vomiting were detected during the trial. The diarrhea occurred in 1 vaccine recipient (PTL-ETEC-003) and 1 placebo recipient, and the vomiting occurred in a placebo recipient.

10 DISCUSSION AND OVERALL CONCLUSIONS

The overall incidences of general symptoms were not significantly different (Fisher's exact test) for subjects receiving either PTL-ETEC-002 or PTL-ETEC-003, compared to placebo recipients. Both attenuated strains, PTL-ETEC-002 and PTL-ETEC-003, were well tolerated with no significant general or gastrointestinal symptoms associated with the administration of either strain. The PTL-ETEC-003 construct was superior to the PTL-ETEC-002 construct in its ability to induce both mucosal and systemic immune responses to CFA/II. PTL-ETEC-003 also exhibited a more sustained intestinal colonization than PTL-ETEC-002. The anti-CFA/II immune responses induced by PTL-003 were comparable after either one or two doses of vaccine, a booster immune response was not evident although a few more seroconvertors were identified after the second dose. The anti-CFA/II immune response induced by PTL-ETEC-003 was comparable to those induced by other candidate ETEC vaccines in clinical trials.

These data indicate that the PTL-003 construct is safe and highly immunogenic and they form the basis for further evaluation of PTL-ETEC-003 in a "proof-of-concept" volunteer challenge study. A toxin-positive ETEC challenge strain (E24377A)homologous in CFA makeup as PTL-ETEC-003 will be validated in an inpatient dose response study (this clinical trial is currently ongoing under BB-IND-9895) and then used to test the protective efficacy of vaccination with PTL-003.

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12 APPENDICES

12.1 Protocol and Protocol Amendments

12.2 Sample Informed Consent

12.3 Sample Case Report Form

12.4 IRB Approval(s) and Correspondence

12.5 Key Study Personnel Curriculum Vitae

12.6 Documentation of Laboratory Certification(s)

12.7 Standard Operating Procedure for Secondary Seed Lot Preparation and FDA Correspondence

APPENDIX 12.1

PROTOCOL AND PROTOCOL AMENDMENTS

APPENDIX 12.2

SAMPLE INFORMED CONSENT

APPENDIX 12.3

SAMPLE CASE REPORT FORMS

APPENDIX 12.4

IRB APPROVAL(S) AND CORRESPONDENCE

APPENDIX 12.5

KEY STUDY PERSONNEL CURRICULUM VITAE

APPENDIX 12.6

DOCUMENTATION OF LABORATORY CERTIFICATION(S)

APPENDIX 12.7

STANDARD OPERATING PROCEDURE FOR SECONDARY SEED LOT PREPARATION AND FDA CORRESPONDENCE

6.16.2 Determination of Sample Size

The number of subjects planned for the study was based on logistical considerations rather than power calculations.

6.17 Protocol Amendments

No formal protocol amendments were made during the conduct of this inpatient protocol. However, because some volunteers experienced abdominal cramps and diarrhea after receipt of the 5 x 10^9 dose level, the third cohort received a reduced dose of 5 x 10^8 bacteria instead of 5 x 10^{10}, as specified in the protocol.

7 STUDY SUBJECTS

7.1 Disposition of Subjects

Twenty-nine (29) subjects were screened and a total of 27 healthy adult inpatient volunteers from the Greater Baltimore area were sequentially enrolled, assigned to receive one of two strains of bacteria at 5 x 10^7 , 5 x 10^9 , and 5 x 10^8 bacteria, as shown in **Table 1** and **Table 2**. Two screened subjects (#18 and #24) withdrew from the study before receiving vaccine, the former for personal reasons and the latter secondary to hyperglycemia.

Table 1
Number of Subjects Planned and Analyzed

# Subjects Planned	# Subjects Screened	# Subjects Completed	# Subjects Withdrew
30	29	27	2

Table 2
Allocation of Treatment to Inpatient Volunteers

Group	PTL-ETEC-002	PTL-ETEC-003	Total
5.7 X10^7	3	3	6
5.7 X10^9	5	6	11
5.7 X10^{8*}	6	4	10

This group replaced the protocol-specified 5 x 10^{10} CFU dose level as adverse events were experienced in the 5 x10^9 CFU group.

Key demographic variables of age, sex, race and treatment group are summarized in **Table 3** and listed by subject in **Appendix 12.8.** Of the 27 healthy subjects, 22 were male and 5 were female; 24 were African-American, 2 were Caucasian, and 1 was Asian, with an age range from 18 to 50 years.

Table 3 Demographics						
Variable[1]	**Tr atment Group**					
	PTL-ETEC-002			**PTL-ETEC-003**		
	5 x 10^7	**5 x 10^8**	**5 x 10^9**	**5 x 10^7**	**5 x 10^8**	**5 x 10^9**
Age (Yrs.)						
Mean	36	41	36	46	27	34
S. D.	9	8	9	2	6	8
Range	28-45	24-46	24-43	44-48	21-34	26-44
Gender						
Male	3	4	4	3	3	5
Female	0	2	1	0	1	1
Ethnicity						
Caucasian	1	0	0	0	0	1
African-American	2	6	5	3	3	5
Asian	0	0	0	0	1	0
Other	0	0	0	0	0	0

[1]Demographics determined at the time of vaccination.

7.2 Protocol Deviations

No formal protocol deviations were documented. In total, 27 of a possible 30 vaccinations were administered. It was thought that these were sufficient numbers exposed to each strain to fulfill the objectives of the trial.

All volunteers attended the required number of outpatient visits as scheduled in the protocol. It is noted that there are study related procedures that were not consistently documented on the case report forms and/or source documents as delineated in the protocol.

7.3 Extent of Exposure

The number of subjects who received treatment is shown in **Table 4**.

Table 4 Treatment Groups			
Group #	**Vaccine Strain**	**Dose**	**# Subjects**
1	PTL-ETEC-002	5 x 10^7	3
	PTL-ETEC-003	5 x 10^7	3
2	PTL-ETEC-002	5 x 10^9	6
3	PTL-ETEC-003	5 x 10^9	6
4	PTL-ETEC-002	5 x 10^{8*}	6
5	PTL-ETEC-003	5 x 10^{8*}	6

* These groups replaced the protocolled 5 x 10^{10} CFU dose level as adverse events were experienced in the 5 x 10^9 CFU groups.

In all cases, the vaccine solution was fully consumed. There were two cases of vomiting, one being mild and within the first 24 hours of vaccination, and one moderate case >24 hours after vaccination (volume for either case was not recorded). The subject with the mild case of vomiting proceeded with eating after vomiting. It is assumed that the vaccine was completely ingested in both cases.

8 EFFICACY EVALUATION

8.1 Data Sets Analysed

There were no subjects for whom pre-vaccination or post-vaccination samples were missing. All available data from the 27 subjects who received a dose of either the live vaccine strain PTL-ETEC-002 or PTL-ETEC-003 were reviewed and samples analyzed.

8.2 Immunogenicity and Efficacy

No direct measurements of efficacy were performed in this study. Response to vaccination was assessed by measurements of antigen specific serum antibodies and by a modified assay for peripheral blood antibody secreting cells. This antibody lymphocyte supernatant (ALS) assay was found to be the more sensitive of the two assays.

Neither IgG nor IgA serum anti-CFA/II antibody responses could be detected in any of the volunteers. The GMT of serum IgG and IgA anti-CFA/II did not change significantly between the sample collected prior to vaccination and those collected after vaccination, although there was great variability between the titers from one volunteer to another. The titers are shown on **Table 5**.

However, anti-CFA responses were seen by ALS assay of supernatant fluids collected from cultured lymphocytes (**Table 5**). Peripheral blood mononuclear cells (PBMLs) collected on Days 0, 7, and 10 were cultured for 48 hours and the culture supernatant subjected to ELISA assay for CFA/II specific IgG or IgA. IgG and IgA titers by ALS increased significantly in the Day 7 sample compared to the pre-immune sample collected on Day 0. By Day 10, the titers decreased (**Table 5**). The clear response in the circulating lymphocytes demonstrates that the vaccine was expressing the CFA antigen in vivo. It is not known if this immune response is protective against infection with ETEC.

Table 5
Immune Serum Antibody Responses to CFA/II Antigen

Strain	Dose	Day	Anti CFA/II Titers: GMT (95% confidence interval)			
			Serum IgG ELISA	Serum IgA ELISA	IgG ALS	IgA ALS
PTL-002	All	0	527(329-845)	256(172-380)	0.141(0.1-0.21)	0.08(0.04-0.15)
		7	531(331-852)	273(174-429)	0.285(0.16-0.52)	1.007(0.38-2.65)
		10	533(332-858)	308(189-503)	0.18(0.12-0.26)	0.1(0.04-0.23)
		14	527(326-855)	274(180-416)	Not Done	Not Done
PTL-002	5×10^7	0	538(75-3882)	336(131-861)	0.158(0.1-0.25)	0.124(0.03-0.5)

Tabl 5
Immune Serum Antibody R sponses to CFA/II Antiq n

Strain	Dose	Day	Anti CFA/II Titers: GMT (95% confidence interval)			
			S rum IgG ELISA	Serum IgA ELISA	IgG ALS	IgA ALS
		7	524(79-3504)	329(57-1883)	0.587(0.14-2.55)	13.2(5.3-33.2)
		10	531(76-3727)	363(121-1088)	0.279(.14-.58)	0.346(0.07-1.67)
		14	512(73-3595)	366(131-1023)	Not Done	Not Done
PTL-002	5×10^8	0	609(325-1143)	180(122-264)	0.11(0.06-0.21)	0.06(0.03-0.14)
		7	594(306-1156)	185(127-270)	0.109(0.06-0.21)	0.203(0.16-0.27)
		10	606(323-1137)	196(114-338)	0.112(0.06-0.2)	0.052(0.02-0.13)
		14	623(312-1248)	188(138-256)	Not Done	Not Done
PTL-002	5×10^9	0	437(278-689)	332(145-760)	0.175(0.08-0.36)	0.087(0.03-0.29)
		7	466(281-775)	402(170-953)	0.585(0.37-0.93)	1.464(0.48-4.43)
		10	459(271-779)	401(163-986)	0.245(0.16-0.37)	0.105(0.02-0.55)
		14	438(283-681)	360(143-907)	Not Done	Not Done
PTL-003	All	0	355(230-548)	141(83-242)	0.083(0.05-0.13)	0.102(0.05-0.02)
		7	383(256-572)	190(117-308)	0.325(0.16-0.64)	0.749(0.35-1.6)
		10	389(249-605)	201(124-326)	0.194(0.11-0.34)	0.388(0.17-0.86)
		14	354(235-533)	181(115-286)	Not Done	Not Done
PTL-003	5×10^7	0	365(84-1588)	470(320-688)	0.059(0.02-0.19)	0.235(0.07-0.77)
		7	384(91-1617)	502(320-790)	0.143(0.06-0.36)	0.342(0.14-0.83)
		10	359(83-1555)	503(325-777)	0.233(0.11-0.49)	0.471(0.15-1.45)
		14	348(82-1474)	467(255-855)	Not Done	Not Done
PTL-003	5×10^8	0	331(165-662)	128(70-234)	0.097(0.08-0.11)	0.057(0.02-0.13)
		7	366(193-696)	142(70-290)	0.148(0.04-0.58)	0.304(0.07-1.29)
		10	91(4.5-1832)	129(52-315)	0.063(0.04-0.1)	0.054(0.03-0.11)
		14	335(162-690)	133(70-250)	Not Done	Not Done
PTL-003	5×10^9	0	366(202-663)	83(40-173)	0.088(0.04-0.22)	0.1(0.03-0.31)
		7	393(235-655)	142(70-288)	0.83(0.51-1.35)	2.025(0.96-4.27)
		10	394(231-674)	159(85-297)	0.246(0.11-0.54)	0.939(0.52-1.69)
		14	371(227-605)	138(73-261)	Not Done	Not Done

9 SAFETY EVALUATION

9.1 Extent of Exposure

All 27 subjects who received a dose of either the live vaccine strain PTL-ETEC-002 or PTL-ETEC 003 according to protocol were included in the analyses.

9.2 Bacteriology

9.2.1 Excretion of the Vaccine Strains

From a maximum of 270 protocolled stool samples/rectal swabs (scheduled daily for the first 6 days after each vaccination), 256 (95%) were collected for culture.

Up to two stool specimens were collected each day after the immunization and were cultured on MacConkey agar and on MacConkey agar with streptomycin. Colonies that grew on the Mac-strep plate were presumed to be vaccine strains and up to ten colonies were spotted onto Luria agar and onto minimal media (Davies). Control (wild type) strains of *E. coli* grow on both of these agars, but the vaccine

strains do not grow on the minimal media. At least one colony of the vaccine strains was saved on nutrient agar slants.

Tables 6A-6C described the excretion of the vaccine strains. Among those receiving a dose of 5 x 10^7 CFU, the vaccine was recovered from the stools of all of 6 volunteers at some time. It was recovered the same day as vaccination from two volunteers and continued to be excreted for up to four days in two volunteers. Of those who received the 5 x 10^8-dose level, 9 of 10 volunteers excreted the vaccine strain at some time, but one volunteer never excreted the strain. Again, some volunteers continued to excrete for up to four days. Of those who received a dose of 5 x 10^9, all of 11 volunteers excreted the vaccine strain and all continued to excrete for four days, compared to only 4 of 16 who received lower doses who continued to excrete for four days ($p<0.0001$, Fisher's Exact Test). There was no difference in the frequency or duration of the excretion of the two vaccine strains when given at comparable doses.

Table 6A

Excretion of Vaccine Strain Following Oral Immunization 5 x 10^7 CFU

Date	Specimen #	Volunteer #1	Volunteer #2	Volunteer #3	Volunteer #4	Volunteer #5	Volunteer #6
		PTL-ETEC-002[a]			PTL-ETEC-003[a]		
27-Oct	1	N		N	Y	N	
	2			Y			
28-Oct	1	Y	Y		Y	Y	N
	2	Y			Y	Y	
29-Oct	1	Y	Y	Y		Y	Y
	2	Y				Y	
30-Oct	1	N	Y	N	N	Y	Y
	2	N			N	Y	
31-Oct	1	N	N	N	N	Y	Y
	2	N		N			
1-Nov	1	N	N	N	N	N	N
	2	N	N	N	N	N	N

[a]Date of immunization: 27 October 1999; Antibiotic started on 31 October 1999 (Y=yes and N=no)

Table 6B

Excretion of Vaccine Strain Following Oral Immunization 5 x 10^8 CFU

Date	Spec #	Vol#19	Vol#20	Vol#21	Vol#22	Vol#23	Vol#25	Date	Spec #	Vol#26	Vol#27	Vol#28	Vol#29
		PTL-ETEC-002[a]						PTL-ETEC-003[b]					
20-Jan	1		Y	Y		N	N	23-Feb	1		Y	N	
	2		Y	N					2			N	
21-Jan	1	N	Y	Y	Y	Y	N	24-Feb	1	Y	Y	Y	Y
	2			Y	Y		N		2				
22-Jan	1		Y	Y	Y	N	N	25-Feb	1		Y	N	

Tabl 6B

Excr tion of Vaccine Strain Following Oral Immunization 5 x 10^8 CFU

Date	Sp c #	V l#19	Vol#20	Vol#21	V l#22	V l#23	V l#25	Date	Spec #	V l#26	V l#27	V l#28	V l#29
		PTL-ETEC-002[a]								PTL-ETEC-003[b]			
	2					N	N		2			N	
23-Jan	1	Y	N	N	N			26-Feb	1	Y	Y	N	Y
	2		Y		N	N	N		2			N	
24-Jan	1	Y	Y	N	N	N	N	27-Feb	1	N	N	N	Y
	2		N		N	N			2	Y	N	N	
25-Jan	1	N	N	N	N	N	N	28-Feb	1	N	N	N	N
	2	N	N	N	N	N	N		2	N	N		N
28-Jan	FU	N	N	N	N	N	N	4-Mar	FU	N	N	N	N
								9-Mar	FU	N	N	N	N

[a]Date of immunization: 20 January 2000; Antibiotic started 24 January 2000 (Y=yes and N=no)
[b]Date of immunization: 23 February 2000; Antibiotic started 27 January 2000 (Y=yes and N=no)

Table 6C

Excretion of Vaccine Strain Following Oral Immunization 5 x 10^9 CFU

Date	Spec #	Vol#7	Vol#9	Vol#10	Vol#11	Vol#12	Vol#8	Vol#13	Vol#14	Vol#15	Vol#16	Vol#17
		PTL-ETEC-002[a]					PTL-ETEC-003[a]					
17-Nov	1	N	N	N	N			N		N	N	
	2			N				N			N	
18-Nov	1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	2	Y	Y			Y	Y	Y	Y	Y		Y
19-Nov	1	Y	Y		Y	Y	Y	Y	Y	Y	Y	Y
	2		Y		Y	Y	Y	Y	Y			
20-Nov	1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	2					Y	Y	Y				
21-Nov	1	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
	2	Y	Y		Y	Y	N	Y		Y		Y
22-Nov	1	N	N	N	N	N	N	N	N	N	N	Y
	2	N	N	N	N	N	N	N	N	N	N	Y
23-Nov	1											N
	2											N
25-Nov	FU	N	N	N	N	N	N	N	N	N	N	N

[a]Date of immunization: 17 November 1999; Antibiotic started on 21 November 1999 (Y=yes and N=no)

9.3 Reactogenicity

9.3.1 Summary of Symptoms

For the assessment of reactogenicity, all signs and symptoms of grade 1 or more were reviewed. Signs assessed include ill appearance, rash, abdominal tenderness, liver palpable or spleen palpable. The signs were assessed as yes=present or no=not present. Symptoms assessed include feels ill, poor appetite, nausea, vomiting, abdominal gurgling, gas, abdominal cramps, diarrhea, tenesmus, chills, malaise, bedridden,

headache, lightheaded, and muscle aches. The symptoms were graded as 0=none, 1=mild; elicited on questions, 2=moderate; self reported, 3=severe; symptoms interfere with normal function.

The number of symptoms recorded following immunization is provided in **Table 7**. No subject experienced the same symptom on more than one separate occasion, however, a subject may have experienced more than one symptom and these are counted. A detailed description of the clinical signs and symptoms experienced by study subjects are described in **Appendix 12.9**.

5 x 10^7 bacteria: Three volunteers received strain PTL-ETEC-002 and three volunteers received strain PTL-ETEC-003. No significant adverse events were seen and the study proceeded to the next highest group.

5 x 10^9 bacteria: Five volunteers received strain PTL-ETEC-002 and six volunteers received strain PTL-ETEC-003. Five of eleven subjects (45%) experienced adverse events in this dose group. Moderate cramps 2/5 (40%), grade 3 diarrhea (545 grams), and moderate vomiting 1/5 (20%) was seen in subjects who received strain PTL-ETEC-002, and moderate cramps in 2/6 (33%), and grade 3 diarrhea (396 grams) was seen in subjects who received strain PTL-ETEC-003 at this dose. Therefore the original planned dose of 5 x 10^{10} bacteria was not administered and instead a reduced dose of the 5 x 10^8 bacteria was administered.

5 x 10^8 bacteria: Six volunteers received strain PTL-ETEC-002 and four volunteers received strain PTL-ETEC-003. Two of six subjects (33%) who received strain PTL-ETEC-002 experienced adverse events; one subject had moderate cramps and another subject had mild vomiting and moderate cramps.

None of the volunteers developed an elevated temperature. In neither case of diarrhea, vomiting, or cramps did the volunteers require restricting or changing activities.

Table 7
Summary of Symptoms Following Immunization

Strain	Dose	Any symptoms	Diarrhea	Cramps	Vomiting
PTL-ETEC-002	5 x 10^7	0/3	-	-	-
	5 x 10^8	3/6	0/6	2/6	1/6
	5 x 10^9	3/5	1/5	2/5	1/5
PTL-ETEC-003	5 x 10^7	0/3	-	-	-
	5 x 10^8	0/4	-	-	-
	5 x 10^9	2/6	1/6	2/6	0/6

9.3.2 Fever

A fever was defined as the occurrence of an oral temperature ≥38.0°C sustained in at least two occasions four hours apart. Where oral temperatures of ≥38.0°C were recorded on two occasions four hours apart prior to Day 4, appropriate cultures were obtained and ciprofloxacin (500mg BID) was prescribed. If no symptoms developed following vaccination, the volunteers were given ciprofloxacin (500mg BID) for 3 days beginning on Day 4.

No fevers were recorded in subjects who ingested the vaccine.

9.3.3 Diarrhea

All stools were examined, graded and weighed by the nurse. The first two stools each day were to be sampled for microbiological examination and tested for occult blood. The stool consistency was graded as 1=formed, 2=soft/mushy, 3=thick liquid, 4=opaque watery, 5=rice in water.

Diarrhea was defined as two or more loose stools (≥ grade 3 stools) in a period of 24 hours totalling 200 grams, or the occurrence of a single loose stool with a weight of 300 grams or more.

Dysentery was defined as the occurrence of diarrhea with blood in the stool as detected as grossly visible blood.

A total of two single episodes of diarrhea were observed in two subjects. The two subjects who experienced diarrhea in the 5 x 10^9 bacteria dose group, one received PTL-ETEC-002 and one received PTL-ETEC-003 and are summarized in **Table 8**. Both had only a single loose stool ≥ 300 grams in weight.

Table 8
Summary of Diarrhea as Defined per Protocol

Volunteer#	Vaccine Strain	Dose	Onset Day Post – vaccination	Duration (hours)	Number of loose stools
8	PTL-003	5 x 10^9	5	17	1
10	PTL-002	5 x 10^9	1	8	1

Diarrhea was not associated with a positive stool culture, thus raising questions about its association with vaccination.

9.3.4 Vomiting

A total of two single episodes of vomiting were recorded. Two subjects experienced vomiting, one in the 5 x 10^8 bacteria dose group and one in the 5 x 10^9 bacteria dose group, both received PTL-ETEC-002 and are summarized in **Table 9**. There was no apparent pattern with respect to timing of vomiting and the administration of vaccine. Subject #10 and subject #21 both experienced vomiting within 24 hours of vaccination.

Subject #21 did not report any symptoms until about 6 hours post vaccination, then suddenly vomited and within 30 minutes of the vomiting episode resumed eating supper. On the basis of these data it cannot be concluded that there is a causal relationship between the ingestion of the vaccine and the occurrence of vomiting.

Table 9 Summary f PTL-ETEC-002 Vomiting			
Volunte r#	Dose	Onset Dav Post-vaccination	Number of episodes
10	5×10^9	< 24 hours	1
21	5×10^8	6 hours	1

9.3.5 Adverse Events

Apart from those specified above, no other symptoms were specifically recorded, with the exception of Volunteer #10 who also reported symptoms of feeling ill and a poor appetite at the same time.

9.3.6 Deaths and Other Serious Adverse Events

There were no deaths or serious adverse events reported during the course of this protocol.

9.3.7 Clinical Laboratory Evaluation

Clinical laboratories were obtained at screening for evaluation of inclusion and exclusion criteria in this protocol. Post-vaccination hematology and clinical chemistry data were not required per protocol.

9.3.8 Safety Conclusions

Two live oral vaccine strains PTL-ETEC-002 and PTL-ETEC-003 were given to inpatient volunteers in the GCRC inpatient ward of the Johns Hopkins Hospital by the Vaccine Testing Unit at Johns Hopkins Bloomberg School of Public Health. They were given increasing doses of the two vaccine strains from 5×10^7 up to 5×10^9 CFU per dose using a bicarbonate buffer to neutralize stomach acid. In general the vaccines were well tolerated; however, gastrointestinal symptoms that were self reported as moderate (grade=2) were seen in those who received doses of 5×10^9 including cramps, diarrhea and one case of vomiting. Some mild to moderate gastrointestinal symptoms were seen in those who received PTL-ETEC-002 dose 5×10^8 including cramps and one case of mild vomiting. None of the symptoms restricted activities nor were they considered serious. The symptoms were not seen in the volunteers who received either 5×10^7 or 5×10^8 CFU doses of PTL-ETEC-003.

10 DISCUSSION AND OVERALL CONCLUSIONS

The two strains of vaccine were in general well tolerated, the few reported symptoms were of mild or moderate severity, no severe signs or symptoms were reported. The lack of a placebo control in this trial made it impossible to determine whether the reported gastrointestinal symptoms could be attributed to vaccine or if they were a

result of ingestion of the bicarbonate buffer used for delivery. Future trials would include a placebo group and an alternative buffer will be used to neutralize stomach acid.

A modified assay to detect antibody-secreting cells in the peripheral blood was found to be far more sensitive than an assay for serum antibodies. Neither IgG nor IgA serum anti-CFA I antibody responses were detected in any of the volunteers. Anti-CFA responses were seen by the ALS assay which measures antibodies secreted from cultured peripheral blood lymphocytes, this response peaked on Day 7 post vaccination and returned to near baseline by 10 days after vaccination.

These data suggest that both vaccine strains are well tolerated and the data form a basis for further evaluation of PTL-ETEC-002 and PTL-ETEC-003 in an outpatient study. The outpatient study should include an assessment of duration of excretion and a placebo control group to better assess the relation of symptoms with the vaccines.

The lymphocyte antibody response should also be continued in the outpatient study, as it appeared to be a more sensitive immune response indicator than serum antibodies. The ALS assay should be compared to the ASC assay for measuring the musocal immune response to the vaccines.

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12 APPENDICES

12.1 Protocol and Protocol Amendments

12.2 Sample Informed Consent

12.3 Sample Case Report Forms

12.4 IRB Approval(s) and Correspondence

12.5 Key Study Personnel Curriculum Vitae

12.6 Documentation of Laboratory Certification(s)

12.7 Standard Operating Procedure for Secondary Seed Lot Preparation and FDA Correspondence

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12.9 Summary of Signs and Symptoms

APPENDIX 12.1

PROTOCOL AND PROTOCOL AMENDMENTS

APPENDIX 12.2

SAMPLE INFORMED CONSENT

APPENDIX 12.3

SAMPLE CASE REPORT FORMS

APPENDIX 12.4

IRB APPROVAL(S) AND CORRESPONDENCE

APPENDIX 12.5

KEY STUDY PERSONNEL CURRICULUM VITAE

APPENDIX 12.6

DOCUMENTATION OF LABORATORY CERTIFICATION(S)

APPENDIX 12.7

STANDARD OPERATING PROCEDURE FOR SECONDARY SEED LOT PREPARATION AND FDA CORRESPONDENCE

APPENDIX 12.8

DEMOGRAPHIC DATA